

Novel Multi-Marker Screening Platform

ImQuest has developed a multi-parameter microtiter assay to expedite the screening of antiviral agents against multiple markers of hepatitis B virus (HBV) replication and to gain insight into mechanism of action. The assay can be customized to meet your development needs.

The multi-marker assay has been optimized for standard cell lines – HepG2 2.2.15, Hep AD38 and Hep AD79 – and also for primary human hepatocytes (PHH). PHH have been pre-screened for their ability to support HBV infection and replication. Cryoplateable PHH are available and all PHH lots are tested for viable infection prior to use at variable MOIs.*

Numerous qPCR and ELISA-based endpoints are currently available including quantification of the following:

- Extracellular HBV DNA
- Intracellular HBV DNA
- cccDNA
- Pre-genomic RNA
- HBsAg, HBeAg, HBcAg

Our suite of anti-HBV services includes assays to evaluate range of action, time of addition assessment, efficacy in drug combinations, potential for resistance, and characterization of resistant strains and effect of serum binding effects.

High Titer HBV Virus Stock Production

ImQuest has the capability to produce HBV stocks and can routinely achieve virus concentrations of approximately 5×10^{10} genome equivalents per milliliter. Virus Production Services include:

- Production of crude virus preparations
- Concentration of virus from crude preparations utilizing Tangential Flow Filtration (TFF)
- Isolation of viral proteins, RNA & DNA
- Determination of virus concentration and infectivity

*Multiple PHH lots have been screened for their ability to support HBV replication. Cell lots are subject to availability; multiple lots may need to be tested prior to initiation of screening studies.

Primary Human Hepatocyte Screening

ImQuest has developed a robust, and reproducible microtiter based screening assay using Primary Human Hepatocytes (PHH). While the stable-cell line assays remain a reliable and robust assay for screening, utilizing chronically infected cells prevents the assessment of agents that inhibit the early stages of viral infection (entry, un-coating and cccDNA formation). Infected PHH can be used to screen for antiviral compounds targeting multiple markers of HBV infection. Timing of infection and compound addition can be customized, and has proved critical to the assessment HBV infection markers. The PHH system more closely resembles the natural HBV infection and remains a gold standard for antiviral agent screening.

Determination of EC₅₀ of Tenofovir Disoproxil Fumarate in HBV-infected PHH

| Marker | TDF EC ₅₀ (μM) |
|-----------------------|---------------------------|
| Extracellular HBV DNA | 0.915 |
| Intracellular HBV DNA | 0.032 |
| HBV cccDNA | > 10.0 |
| HBV pgRNA | < 0.032 |
| Extracellular HBsAg | > 10.0 |
| Extracellular HBeAg | > 10.0 |

Determination of EC₅₀ of IFNα-2a in HBV-infected PHH

| Marker | IFNα-2a EC ₅₀ (U/mL) |
|-----------------------|---------------------------------|
| Extracellular HBV DNA | < 3.16 |
| Intracellular HBV DNA | 24.2 |
| HBV cccDNA | < 3.16 |

Using state-of-the-art and established *in vitro*, *ex vivo* and *in vivo* models, ImQuest BioSciences helps our clients rapidly identify antiviral agents with the potential to inhibit the replication of viruses.

Antiviral Services

- Determination of efficacy and toxicity in established and fresh human cells
- Range of action assays, including:
 - Efficacy evaluation in a variety of phenotypically distinct human cell types
 - Efficacy evaluation against drug-resistant virus isolates
 - Efficacy evaluation in chronic and latent models of infection
 - Cell-to-cell virus transmission assays
 - Virucidal activity evaluation
 - Effect of serum and serum protein on antiviral activity
 - Effect of time-of-drug addition and viral multiplicity of infection
 - Efficacy against a range of infectious viruses
- Mechanism of action evaluations using cell-based, molecular/biochemical and enzymatic assays
- Combination therapy evaluation with other FDA-approved and experimental inhibitors
- Drug-resistant virus selection and characterization analysis
- Pharmaceutical product characterization and formulation

About ImQuest BioSciences

ImQuest BioSciences is a preclinical contract research and development company that evaluates the potential of new and novel pharmaceutical products. We specialize in services for the development of drugs, vaccines and biologics for the treatment and prevention of infectious disease, cancer and inflammatory disease.

Virus Panels

Human Immunodeficiency Viruses

- Hundreds of clinical and laboratory HIV-1 strains representative of Group M and O
- Drug-resistant and multi-drug resistant HIV-1 isolates
- Clinical and laboratory HIV-2 strains

Hepatitis and Mosquito Borne Viruses

- Hepatitis B Virus
- Hepatitis C Virus surrogate - Bovine Viral Diarrhea Virus (BVDV)
- Hepatitis C Virus replicon system
- Hepatitis A Virus
- Zika Virus
- Chikungunya Virus
- West Nile Virus
- Dengue Virus subtypes 1 through 4
- Yellow Fever Virus

Respiratory Viruses

- Influenza Virus (A & B) - numerous wild-type and drug-resistant strains
- Human Parainfluenza Virus
- Respiratory Syncytial Virus
- Human Rhinovirus
- Measles Virus
- Human Adenovirus

Herpes Viruses

- Herpes Simplex Virus type 1 and 2
- Human Cytomegalovirus
- Varicella Zoster Virus

Enteric Viruses

- Enterovirus
- Echovirus
- Coxsackie Virus
- Poliovirus